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BRILLOUIN SPECTROSCOPY DATA BASE FOR BIOLOGICAL THREATS

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Brillouin scattering from biological materials is conducted using a tandem Fabry-Perot interferometer. The interferometer is uniquely designed to measure small frequency shifts that are characteristic of Brillouin scattering. Brillouin scattering from DNA, ovalbumen, the *Bacillus* spores *globigii* and *thuringensis* were measured to determine the feasibility of biological material discrimination using Brillouin scattering. It was found that all biological materials exhibited unique Brillouin spectra and that it was possible to discern *Bacillus* spores at the species level. It is concluded that Brillouin spectroscopy has great potential as an optical tool for the detection and discrimination of threat biological materials. The only limitation in this approach is that the cross sections are small, and the tool is best suited for either point detection or short-distance remote detection.

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PREFACE

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BRILLOUIN SPECTROSCOPY DATA BASE
FOR BIOLOGICAL THREATS

1.   INTRODUCTION

The remote detection and identification of threat biological aerosols is a critical military need that enhances the survivability of the field soldier. The Joint Biological Standoff Detection System (JBSDS) Block II program has a standing objective to develop a standoff biological detection system that can detect and discriminate biological aerosols at a concentration of 1000 particles per liter of air at 1 kilometer. To address this requirement, a Defense Technology Objective, DTO CB.35, Standoff Biological Aerosol development program was funded to seek out relevant technologies that address the requirements of the JBSDS Block II program.

Recently considerable work has been conducted on the use of ultraviolet (UV) radiation to detect biological aerosols (Faris et al., 1997). Their work showed that when biological spores, such as *Bacillus globigii* (BG) and *thuringensis* (BT), are excited with 266 nm radiation, the spores exhibit a fluorescence emission spectra that is characterized by a broad peak centered near 325 nm. This emission peak is indicative of multimode excitation of the amino acids tryptophan and tyrosine (Lakowicz, 1983). Because of the broad, featureless nature of the emission spectra of the biological spores, it is a challenge to develop algorithms that permit the discrimination of biological organisms at the species level. In addition, the fluorescence emission of some background aerosols, such as pollens, have similar spectra to the biological spores further confounding the problem of developing biological discrimination methodologies based on UV fluorescence. Evidently an alternate technology may be necessary to achieve species-level discrimination of biological aerosols.

During the late 1970's work was done on Brillouin scattering from DNA films and fibers where it was demonstrated that DNA material exhibited unique frequency shifts on the order of 10 to 30 GHz (Maret et al., 1979). They concluded that at least one of the two shifts corresponded to the activation of acoustic phonons traveling parallel to the DNA orientation axis. Because this shifted line showed the same polarization as the exciting beam, they attributed the shifted peak to scattering from longitudinal phonons. Engberg et al. (1999) studied Brillouin scattering from poly(propylene glycol), PPG, polymer chains of varying length and with different end groups. They argued that inelastic Brillouin scattering resulted in frequency shifts that were related to the velocity of sound in the medium by the relation

$$\omega = v_s q \qquad (1)$$

where q is the magnitude of the change in the wave vector upon scattering and can usually written as,

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$$q = 2k_o \sin (\theta/2), \qquad\qquad (2)$$

where θ is the scattering angle.

The acoustic phonon velocity (speed of sound) is related to the material elastic constant C and density ρ by the equation,

$$v_s = \sqrt{C/\rho} . \qquad\qquad (3)$$

This relationship establishes the fact that materials of different elasticity and density should exhibit different Brillouin scattering frequency shifts. The set of relational definitions (Equations 1-3) constitute the motivation to apply Brillouin scattering to the problem of the detection and discrimination of biological materials. It is the intent of this study to develop a representative Brillouin spectral database for biological materials of interest and to demonstrate the feasibility of biological discrimination via Brillouin scattering.

2.   EXPERIMENT AND DISCUSSION

All scattering measurements are conducted with a tandem Fabry-Perot interferometer coupled to a solid-state photon detector. The tandem etalons of the interferometer permit the measurement of small frequency shifts that are a characteristic of Brillouin spectra. A 532 nm Nd Yag excitation laser operating at 200 milliwatts is used to excite biological samples deposited on a microscope slide. A polarization rotator is used to analyze the affect of radiation polarization on the Brillouin spectra. Spectra are collected at 90 and near 180 degrees at a temperature of 25 degrees Centigrade. The interferometer uses a unique window-shutter system to eliminate the Rayleigh scattering from the Brillouin signal. Figure 1 shows a schematic of the Brillouin spectroscopy setup.

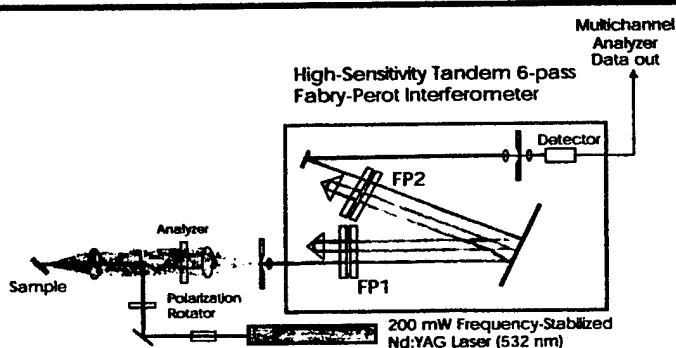# Brillouin Spectroscopy:
Experiment

Figure 1. Schematic of Brillouin Scattering Experiment.

Initial experiments were conducted on the alignment and focusing of the laser beam to maximize the system signal response. Plexiglas was used as a standard material to conduct initial alignment experiments. Optimization procedures primarily focused on the alignment of the optics both external and internal to the Fabry-Perot interferometer. Figure 2 shows the spectrum of Plexiglas obtained in the 180 degree backscatter orientation. The spectrum exhibits two sharp peaks near ± 15.4GHz corresponding to Stokes and anti-Stokes lines attributable to Brillouin scattering.
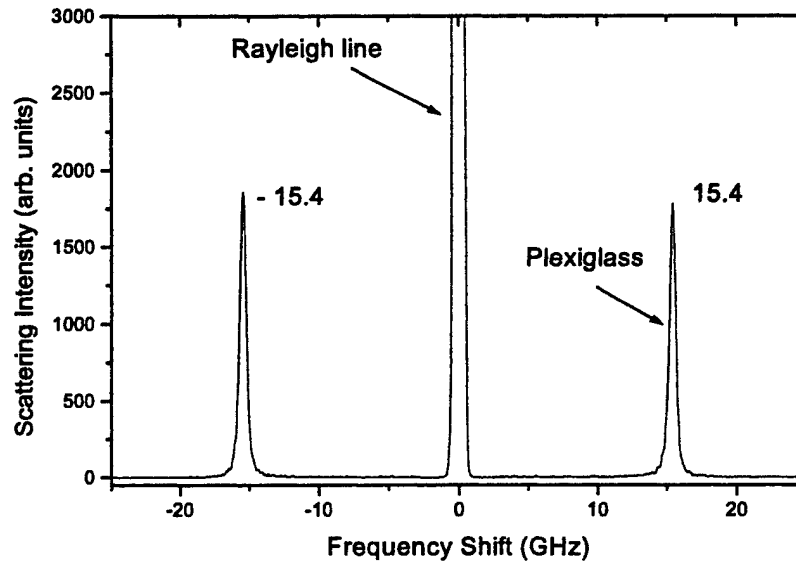

Figure 2. Scattering from a Slab of Plexiglas in the Backscatter Geometry Showing the Stokes and Anti-Stokes Brillouin Frequency Shifts Near 15GHz.

Note the large Rayleigh scattering peak at 0 GHz that results from elastic light scattering from the bulk material. By using a tandem etalon (interference mirrors) arrangement it is possible to resolve frequency separations on the order of 5 GHz and the Plexiglas spectra is easily resolvable. Similar measurements of the Brillouin scattering from glass (re: microscope slides) reveals a frequency shift of 32GHz owing to the greater elasticity of glass ala Plexiglas. Having demonstrated the capability of the Fabry-Perot interferometer to measure accurately the Brillouin scattering from homogeneous solids such as glass and Plexiglas, it was of interest to apply the experiment to more complex materials such as biological solids. The strategy of the study is to measure the Brillouin scattering from progressively more complex biological materials. With this said, the spectra for DNA, ovalbumen, and *bacillus* spores were measured in sequential order. The DNA samples were prepared by depositing the material as a uniform film on a glass microscope slide. Figure 3 shows the Brillouin spectra for calf thymus DNA revealing the clearly visible Stokes and antiStokes peaks at 17.2 GHz. Note that the glass line is clearly evident in the spectra and that the half-width of the DNA line is greater than the glass line. The peak line-width is affected by damping processes in the solid. The greater the damping affects the greater the line half-width.
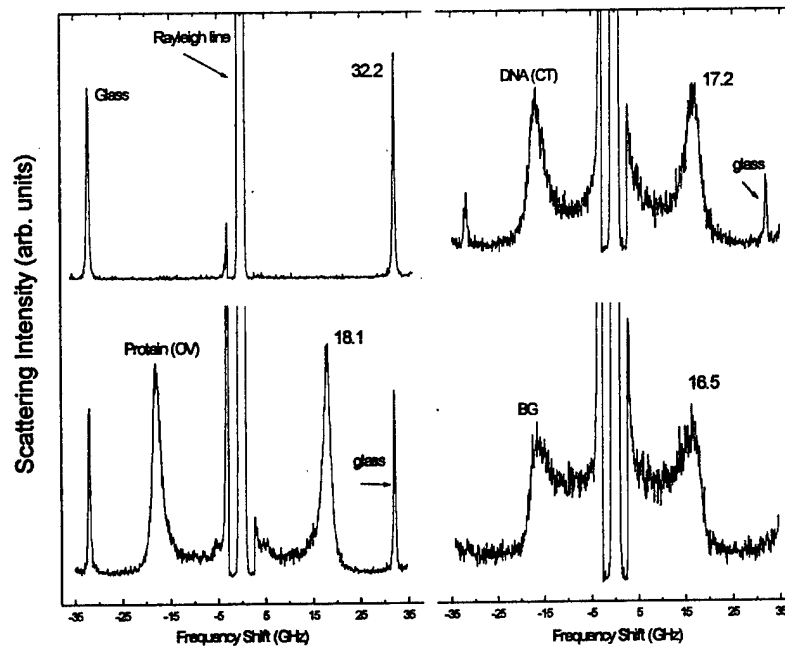
Figure 3. Brillouin Scattering Spectra for DNA, Ovalbumen and *Bacillus* Spores.

Figure 3 also shows the scattering spectra of the protein ovalbumen (OV) and *Bacillus* spores (BG). The respective frequency shifts are 17.2, 18.1, and 16.5 for DNA, ovalbumen, and *Bacillus* spores. As comparison, Maret et al., (1979) measured the Brillouin spectra for films and fibers of DNA. Using a similar optical arrangement as ours, they found that dry semi-crystalline fibers of DNA exhibited a doublet frequency shift one centered at 20 GHz and one at 10 GHz. We offer no explanation for the differences in the DNA Brillouin spectra between our measurements and Maret et al (1979). The remainder of the spectra in Figure 3 shows that each biological material is discernable from each other and that for this class of biological materials discrimination is feasible. Of the spectrum shown in Figure 3, the protein ovalbumen exhibits the greatest elasticity and the *Bacillus* spores exhibit the least elasticity.

A major objective of this study is to determine whether Brillouin scattering could be used to discriminate biological materials at the species level. Figure 4 shows the combined spectra of the two *Bacillus* species *globigii* (BG) and *thuringensis* (BT). It is evident that the Brillouin frequency shift is distinct for the two species of *Bacillus*. Thus it appears that Brillouin scattering can successfully be used to discriminate these biological materials at the species level. Because BT exhibits a greater frequency shift than BG, it is natural to suggest that BT has a greater elasticity than BG. Generally the greater the elasticity, the greater the intermolecular interactions, and it is attractive to surmise that the intermolecular interactions of the BT spores is greater than the BG spores.
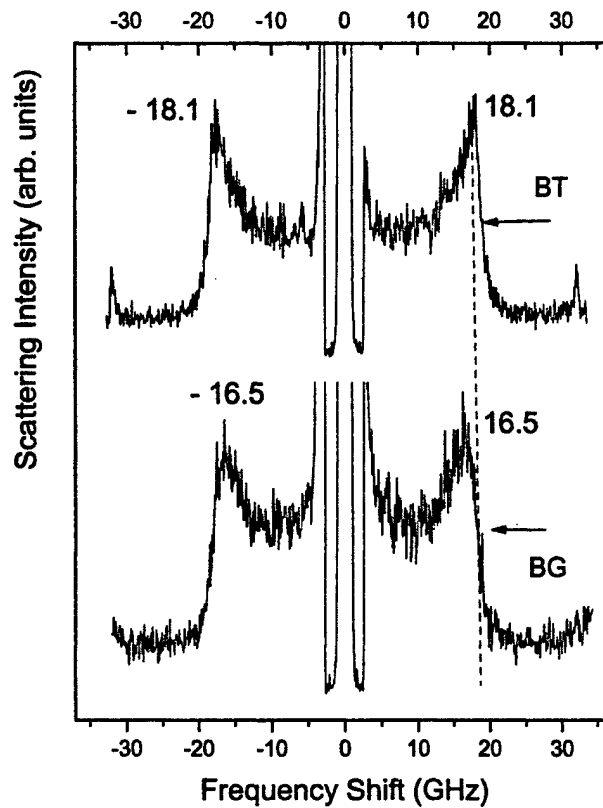
Figure 4. Combined Brillouin Scattering Spectra for *Bacillus globigii* and *thuringensis*.

Differences in the Brillouin spectra of like material have been observed for polypeptide-containing tissues such as collagen and retractor muscle (Harley et al., 1977). They compared the spectra of molluscan muscle to that of collagen, two materials with different polypeptide configurations. It was found that the Brillouin frequency shift differed for the muscle and collagen by approximately 6%. Figure 4 shows that the Brillouin frequency shift difference between BG and BT is approximately 10%. While the work of Harley et al. (1977) does not corroborate the findings of the Brillouin spectral differences for BG and BT spores, it certainly offers a hypothetical justification for these differences.

It is also of interest to determine whether Brillouin scattering can be used to discriminate vegetative from *Bacillus* spores. Figure 5 shows that the shifts
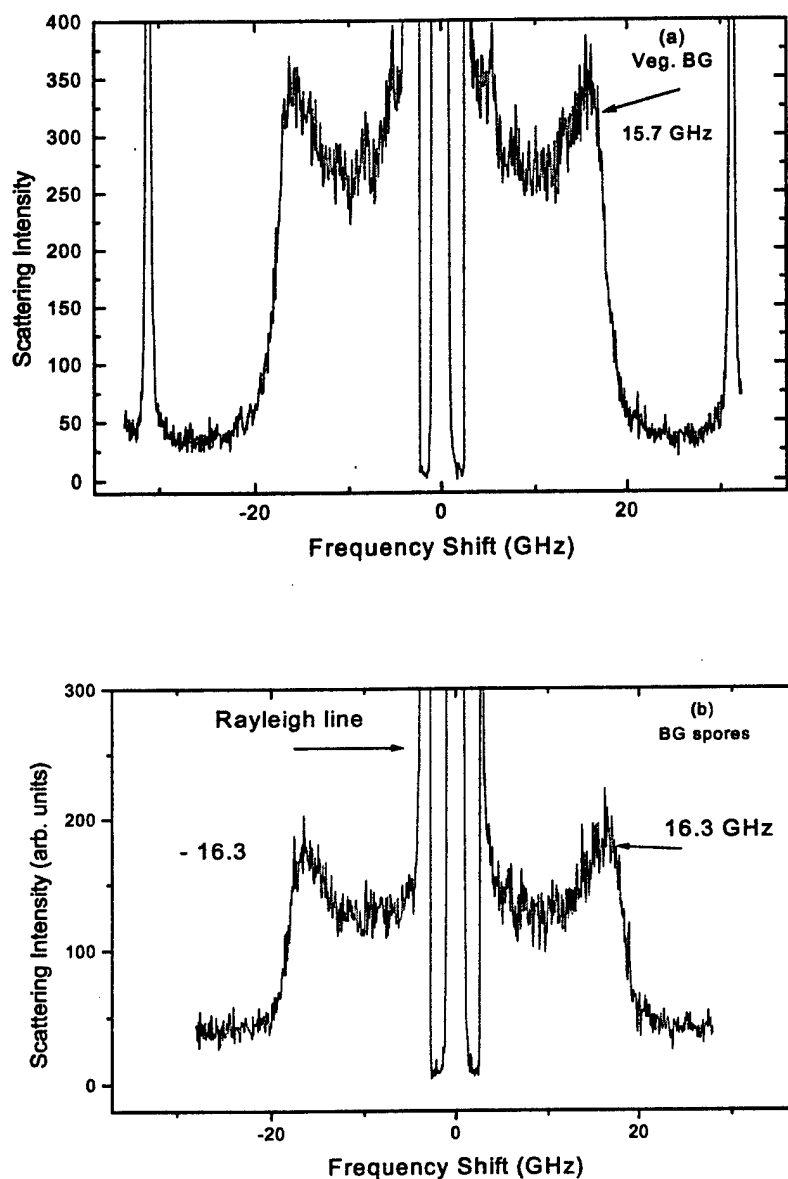

Figure 5. Brillouin Scattering from Vegetative *Bacillus* (a) and *Bacillus* Spores (b).

of vegetative and spore *Bacillus* are 15.8 and 16.3 GHz, respectively. This frequency shift is significant and is outside the range of experimental error. As discussed earlier, the greater the elasticity of the material, the greater the Brillouin frequency-shift. The fact that the vegetative frequency-shift is less than the spore shift could indicate that the spore coat adds rigidity to the biological material. This conclusion is speculative and needs confirmation.
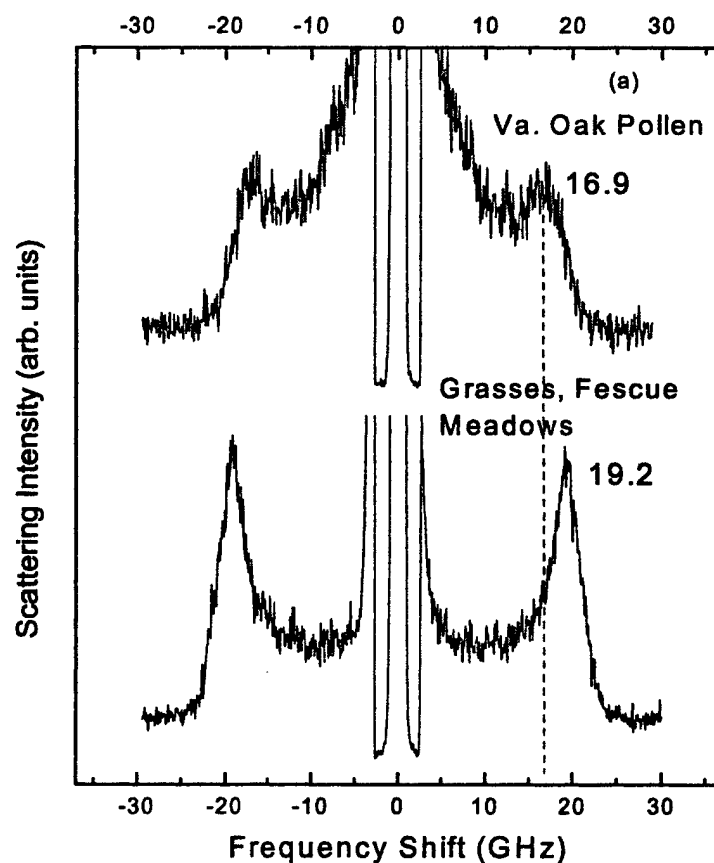
Figure 6. Brillouin Scattering Spectra for Grasses and Pollens.

While the discrimination of biological materials at the species level is significant, another important issue relates to the discrimination of threat biological materials from innocuous background materials such as pollens, molds, and grasses. Figure 6 shows the Brillouin spectra of some important biological background materials. Both pollen and grass exhibit distinct spectral shifts to that of *Bacillus* spores. While not shown, mold also shows a Brillouin spectrum distinct from that of the biological spores. Figure 7 shows the Brillouin spectra of other background interferences not of biological origin.
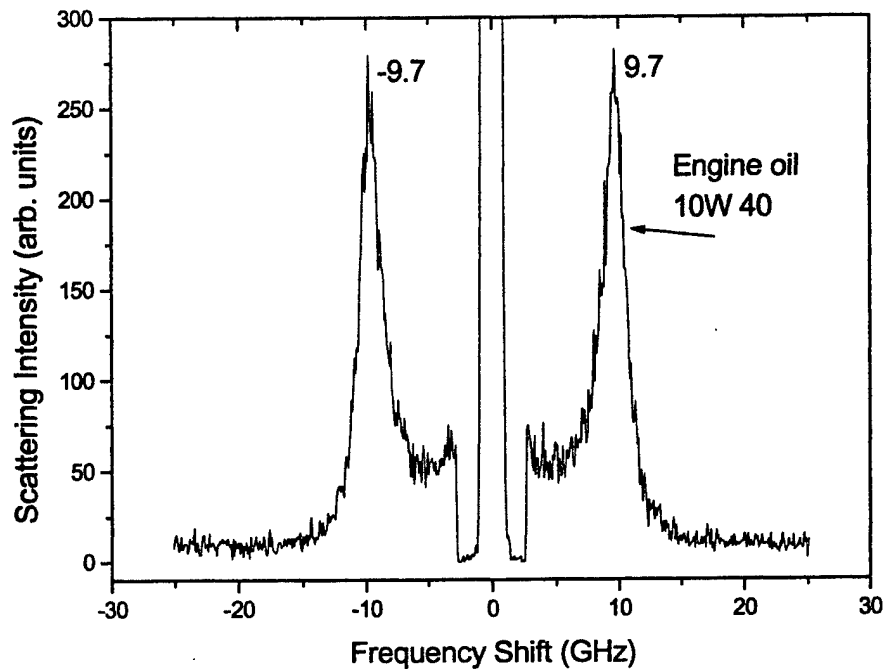
Figure 7. Brillouin Spectrum for Field Background Interference Engine Oil.

3.   CONCLUSIONS

        Brillouin scattering from biological materials is conducted using a tandem Fabry-
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that are characteristic of Brillouin scattering. Brillouin scattering from DNA, ovalbumen, the
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